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## DRAFT TANZANIA STANDARD

# Methods for determination of organic preservatives in foodstuffs – Part 1: benzoic acid and its salts



## TANZANIA BUREAU OF STANDARDS

## 0. Foreword

For protecting food from microbial deterioration, a number of methods as application of heat or cold, dehydration, fermentation, irradiation or addition of certain chemicals are employed. Besides extending the periods of use of food a chemical preservative should be safe for human consumption, should not impart undesirable organoleptic changes, be economical in use and be capable of being analyzed. While the use of preservative to be safe under conditions of use is governed by law, it is considered necessary to prescribe methods for their analysis. The use of these methods would not only ensure repeatable and reproducible results for their correct interpretation, but would also facilitate inter-laboratory comparisons

There are two classes of preservatives, class I and class II. Class I preservatives include common salt, sugar, dextrose, glucose (syrup), wood smoke, spices, vinegar  honey, etc. class II preservatives include inorganic substances such as sulphurous acid including salts thereof, nitrates of sodium or potassium and organic substances like benzoic acid including salts thereof, sorbic acids and including its sodium, potassium and calcium salts and sodium and calcium propionate.

This standard, covering the determination of organic preservatives, is being issued in three parts. This part (Part 1) covers the determination of benzoic acid and its salts in foodstuffs. Part 2 covers propionic acid and its salts and part 3 covers sorbic acid and its salts.

Benzoic acid and its salts are commonly used as preservatives in non-alcoholic wines, squashes, crushes, fruit syrups, cordials, fruit juices, barley water, sweetened mineral water, jam, marmalade, canned cherry, fruit jelly, pickles and chutney made from fruits or vegetables, tomatoes and other sauces, syrups and sharbats.

In the preparation of this standard assistance was drawn from IS 12014 (Part 1):1986, Methods for determination of organic preservatives in foodstuffs – Part 1: benzoic acid and its salts published by Bureau of Indian Standards (BIS)

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with TZS 4

## 1. Scope

This Tanzania standard prescribe the methods for determination of benzoic acid and its salts used as preservatives in foodstuffs

## 2. Normative References

The following referenced standards referred to in the text in such a way that some or all of their content constitutes requirements of this standards.  For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies

*TZS 59: Water for analytical laboratory use – Specification and test method*

*TZS 4: rounding off numerical values*

*TZS 672: automotive gasoline (premium motor spirit)*

## 3. Quality of reagents

3.1 analytical grade chemicals and distilled water conforming to TZS 59 shall be employed in tests.

## 4. General

4.1 this standard specifies three methods for determination of benzoic acids and its salts namely, thin layer chromatography, titration and spectrophotometric methods. Thin layer chromatographic methods shall be used for qualitative detection and titration or spectrophotometric methods shall be used for quantitative estimation of benzoic acid

**4.2 Principle**

benzoic acid acids are isolated from food by extraction with diethyl ether and successive partitioning into aqueous NaOH and $CH_2Cl_2$. Acids are converted to trimethylsilyl (TMS) ester and determined by gas chromatography (GC). Phenyl acetic acid is used as internal standards for benzoic acid

## 5. Thin layer chromatographic (TLC) methods

5.1 **Reagents**

5.1.1 Magnesium sulphate ($MgSO_4.7H_2O$)

5.1.2 Phosphoric Acid

5.1.3 Sodium Hydroxide -1N

5.1.4 concentrated hydrochloric acid – analytical grade

5.1.5 Chloroform

5.1.6 diethyl ether

5.1.7 Solvent – A mixture of n-hexane and acetic acid in the ratio of 96:4

5.1.8 Bromocresol Green

5.1.9 Ethanol – 50 percent and 95 percent

5.1.10 Standard Benzoic Acid

**5.2 Apparatus**

5.2.1 Kjeldahl Flask

5.2.2 separating funnel

5.2.3 Ultraviolet lamp

5.2.4 Ultraviolet Spectrophotometer

5.2.5 TLC Plate - made up of a layer containing kieselguhr GF 254 kieselguhr G (1:1) to a thickness of 0.25 mm. Activate at 100 °C for 1hour

**5.3 Preparation of the Sample**

5.3.1 Liquids – accurately weigh 50 to 60g sample directly in a kjeldahl flask with a small portion of water and proceed;

5.3.2 Solids - accurately weigh 40g into a high-speed blender. Add 100mL distilled water, blend until homogeneous and transfer to a kjeldahl with a small portion of water.

5.3.3 Semisolid and Solid Liquid Mixture - Blend the whole sample in high speed blender to get a homogeneous mixture. Weigh 50 to 60g of the blended sample in a kjeldahl flask with a small portion of distilled water and proceed.

**5.4 Procedure**

5.4.1 Extraction - To the prepared sample (see 5.3), add 200g of magnesium sulphate and 25mL of phosphoric acid. Wash the neck of the flask till the total volume is 350 to 375mL. steam distil the preparation into separating funnel containing 50mL of sodium hydroxide collecting 725 to 750mL of distillate in 90 minutes. Rinse the condenser with 20mL of water. Acidify the distillate to litmus with 20mL of hydrochloric acid. Make the extraction solution by mixing two volume of chloroform and one volume of ether. Then extract the distillate with 100mL and four 50mL portions of the extraction solution, each extraction should be shaken for 1 minute vigorously. Collect the combined extract in a beaker and evaporate carefully on a steam-bath and take in a 25mL volumetric flask

5.4.2 Spot on the plate 100µL of the sample solution with the standard benzoic acid. Place the plate in a chamber containing the solvent and develop up to 10cm. air-dry the plate for 10 minutes. See the benzoic acid spots under the ultraviolet light. For the better visibility, spray the plate with 0.5 percent bromocresol green ethanol. Spots are permanent and can be observed for several days.

5.4.3 Approximate quantitative estimation by visual comparison and quantitative estimation by densitometric comparison of the intensity of the colour of the spot of the sample with those of the standards may be done. Quantitative estimation may also be done by scraping off the spot of the sample in unsprayed condition identified with the help of the ultraviolet light. Trasfer the scrapped sample into centrifuge tube also prepare a blank. To each add 7mL of 95 percent ethanol. Shake and centrifuge. Take supernatant in 5cm microcell. Read in spectrophotometer from 310 to 350nm against the blank. Calculate as under:

Percent benzoic acid x 1.18 = percentage sodium benzoic

## 6. Titration Method

6.1 reagents

6.1.1 Saturated sodium chloride solution

6.1.2 Pulverized sodium chloride

6.1.3 Calcium hydroxide solution 1:3 solution of milk of lime

6.1.4 Dilute hydrochloric acid - 1:3

6.1.5 Chloroform

6.1.6 Ethyl Alcohol - Made Neutral with an alkali using phenolphthalein as an indicator

6.1.7 Phenolphthalein indicator solution - prepared by dissolving 0.1g in 100mL of rectified spirit conforming to TZS 672

**6.1.8 Standards Sodium Hydroxide – 0.05N**

**6.2 Preparation of the Sample**

6.2.1 General Food Stuffs - Weigh accurately about 150g of the material put into digesting tube, (grind if necessary) Add about 300mL of saturated sodium chloride solution. Digest the material and then add 15g of pulverized sodium chloride. Make the solution alkaline to litmus by adding required amount of calcium hydroxide solution. Transfer the solution to a 500mL volumetric flask and make up to volume with saturated solution of sodium chloride. Allow it to stand for at least two hours while shaking frequently; centrifuge if necessary, and filter.

6.2.2 Catsup - weigh accurately about 150g of the sample and add to it 15g pulverized sodium chloride and transfer the mixture to a 500mL volumetric flask rinsing with 150mL saturated sodium chloride solution. Make slightly alkaline to litmus paper with 10 percent sodium hydroxide solution and dilute to volume with saturated sodium chloride solution. Allow it to stand for 2 hours shaking frequently centrifuge, if necessary and filter.

6.2.3 Jellies, Jams Preserves and Marmalades - weigh accurately 150g sample and digest in 300mL of saturated sodium chloride solution. Add 15g of pulverized sodium chloride and make it alkaline to litmus paper with calcium hydroxide solution. Transfer the solution to a 500mL volumetric flasks and make up to volume with sodium chloride solution. Allow it to stand for 2 hours shaking frequently centrifuge, if necessary and filter.

6.2.4 Cider containing alcohol and similar products - weigh accurately 250g sample make it alkaline to litmus paper with 10 percent sodium hydroxide solution and evaporate to steam bath to 100mL. Transfer the solution to a 250mL volumetric flasks and add 30g of pulverized sodium chloride and shake until dissolved. Dilute to volume with saturated sodium chloride solution. Allow it to stand for 2 hours shaking frequently centrifuge, if necessary and filter.

6.2.5 Salted or Dried Fish - Accurately weigh 50g of sample and grind it. Transfer the sample into a 500mL volumetric flask with a little amount of distilled water. Make the solution slightly alkaline to litmus paper with 10 percent sodium hydroxide solution and dilute to volume with distilled water. Allow it to stand for 2 hours shaking frequently centrifuge, if necessary and filter. Pipette 300mL of the filtrate into another 500mL volumetric flask and add 90g of pulverized sodium chloride. Shake until sodium chloride dissolves and dilute to volume with saturated sodium chloride solution. Mix thoroughly and filter off the precipitated protein and other extraneous matter.

**6.3 Procedure**

6.3.1 Pipette accurately 100 to 200mL of the filtrate (see clause 6.2) into a separating funnel. Neutralize it to litmus with hydrochloric acid and add 5mL in excess. Extract with 70, 50, 40, and 30mL of chloroform

shaking with a rotary motion to avoid an emulsion. If an emulsion forms, break up with a glass rod, by drawing off into a second funnel and giving one or two sharp up-and-down shakes or by centrifuging. A neutral hygroscopic reagent like calcium chloride may be used for breaking the emulsion. Avoid drawing off the aqueous layer

6.3.2 Transfer the extract to a porcelain dish rinse the container several times with a small quantity of chloroform, and evaporate at a room temperature in a current of dry air. Alternatively, transfer it into an Erlenmeyer flask and distil slowly to one-quarter of its volume. Transfer it to the dish, rinsing the flask with a small amount of chloroform and evaporate to dryness at room temperature in a current of a dry air. Dry the residue overnight in a desiccator containing concentrated sulphuric acid.

Note – if necessary, the chloroform extract may be washed with distilled water to make it free from hydrochloric acid as the presence of the acid may vitiate the results.

6.3.3 Dissolve the residue in 30 to 50mL of ethyl alcohol, and add 10mL of water and one or two drops of phenolphthalein indicator solution. Titrate this solution with a standard sodium hydroxide solution and note the volume of the sodium hydroxide

**6.4 Calculation**.

$$Benzoic\ Acid\ \left(\ {}^{mg}/_{kg}\ \right) = 61{,}000{,}000\ x\ \frac{N\ X\ V}{V1\ M}$$

Where

N = normality of the standards sodium hydroxide solution

V = Volume of the standards sodium hydroxide solution

$V_1$ = Volume of filtrate taken (6.2); and

M = mass in g, of the material.

## 7. Spectrophotometric Method

**7.1 Reagents**

7.1.1 Crystallized Tartaric Acid

7.1.2 Sodium Hydroxide – 1N

7.1.2 sodium hydrogen carbonate ($NaHCO_3$)

7.1.3 Potassium Dichromate – aqueous solution of 33 to 34g

7.1.4 Dilute Sulphuric Acid – Obtained by diluting two volumes of concentrated sulphuric acid with one volume of water

7.1.5 Diethyl Ether – freshly redistilled

7.1.6 Standard Benzoic Acid Solution – 100mg/L prepared in diethyl ether

**7.2 apparatus and equipment**

7.2.1 Volumetric flask – 50mL capacity

7.2.2 Beakers – 50 and 100mL capacity

7.2.3 Pipette – 20mL

7.2.4 Graduated pipette

7.2.5 Separating funnel

7.2.6 Homogenizer

7.2.7 Ultraviolet spectrophotometer – provided with a 0.5mm monochromator with silica cells of thickness 20mm, fitted with ground lids

**7.3 Procedure**

7.3.1 Preparation of the sample

7.3.1.1 Liquid products – homogenize the sample well

7.3.1.2 Thick products homogenize the sample for analysis after carefully mixing it

7.3.1.3 Solid products – cut a portion of the sample for analysis into small pieces and remove the stones. Take about 40g of the product and crush them in a homogenizer or a motor

**7.3.2 Test Portion**

7.3.2.1 Liquid products – take 20mL of the prepared sample free from suspended matters with the help of the pipette, mix them with about 50mL of distilled water and transfer them into a 500mL separating funnel. In the case of the pulpy liquid product, place 20mL of the product in a mortar and dilute them with 20mL of distilled water. After decantation, filter the liquid. The residue is dissolved twice with 20mL of water. The filtrates are directly collected in the separating funnel.

7.3.2.2 Thick or solid products – weigh to the nearest 0.01g, about 10g of the prepared sample. Entrain in a 250mL borosilicate glass flask with ground-glass stopper with 30 to 40mL of distilled water; add about 50mg of sodium hydrogen carbonate (see note), shake and place on a water bath at 70 to 80°C for 15 to 30 minutes. Pour the contents of the flask on a fluted filter paper and rinse twice with 15 to 20 mL of distilled water. The filtrate and the washing are collected in a 500mL separating funnel. Allow to cool.

NOTE - The purpose of adding sodium hydrogen carbonate ($NaHCO_3$) is to salify the benzoic acid, the traces of which could be lost by volatilization

7.3.2.3 Extraction of the benzoic acid - introduce 1g of tartaric acid into the separating funnel containing the aqueous dilution of the product to be analyzed (see 7.3.2.1 and 7.3.2.2), add 60mL of diethyl ether and shake carefully.

Allow to settle and then collect the other layer in another separating funnel. Wash the aqueous phase in the first funnel a second time with 60mL diethyl ether. Allow to settle and add once again the other layer to the first one. Carry out in the same manner a third extraction with 30mL of diethyl ether.

Add successively 10mL, then 5mL of sodium hydroxide solution and two sets of 10mL of distilled water to the combined ether extract. Shake and after allowing to settle down, collect the aqueous phase in a dish. Place the dish on a water-bath until about half of the alkaline solution is evaporated so as to eliminate the dissolved diethyl ether.

7.3.2.4 Purification of benzoic acid - after cooling, transfer the contents of the dish to a 250mL flask with a ground-glass stopper containing a mixture of 20mL of dilute sulphuric acid and 20mL of potassium dichromate solution. Stopper the flask, shake and allow to remain in contact for at least an hour.

NOTE1 – the extension of time of this oxidation does not create any difficulty because the benzoic acid withstands this oxidation mixture

NOTE2 – when the original product also contains sorbic acid it necessary to extend by 24 hours the oxidation in order to ensure the complete elimination of this anti-fungal agent.

7.3.2.5 Preparation of the ether solution – extract the benzoic acid by treating twice the chromic solution with 20 to 25mL of diethyl ether solutions. Wash the ether solution twice with a few millilitre of distilled water. After carefully decanting on a dry filter paper, collect the filtrate in a 50mL volumetric flask. Wash the filter with a few millilitre of diethyl ether so as to bring the filtrate to 50mL

7.3.2.6 Determination – place the diethyl ether solution in a silica cell with a ground lid of thickness 20mm and measure the absorbance of this solution with respect to the absorbance of the pure diethyl in a similar silica cell.

The differential measurement of the emergence at 272nm is calculated as follows:

$$B = \frac{A+C}{2}$$

Where

B = absorbance at 272 nm,

A = absorbance at 267.5 nm,

C = absorbance at 276.5 nm

7.3.2.7 plotting of the calibration curve – into a series of six 50mL volumetric flasks, introduce 5, 7.5, 10, 12.5, 15 and 20mL of the standard solution of benzoic acid. Make up the volume to 50mL with diethyl ether. The solutions obtained contain 10, 15, 20, 25, 30 and 40µg of benzoic acid per litre. Carry out on the determinations on these solutions the differential measurements by proceeding as indicated in 7.3.2.6.

Plot the curve giving the differential measurements as a function of the number of micrograms of benzoic acid per litre indicated above.

**7.4 Expression of the Results**

7.4.1 Liquid Products – ‘‘Benzoic acid’’ $^{mg}/_{l} = \frac{M_2 \ x \ 50}{20}$

Where;

$m_2$ = mass in milligram of benzoic acid read on the calibration curve.

The results can also be expressed in milligrams per kilogram

7.4.2 Thick or Solid Products – “Benzoic acid’’, $\frac{mg}{kg} = \frac{m_2 \ x \ 50}{m_1}$

Where

$m_1$ = mass in grams of the product contained in the test portion, and

$m_2$ = mass expressed in milligram of benzoic acid read on the calibration curve